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Non-medicated milk replacer containing Bacillus amyloliquefaciens reduces intestinal pathogenic bacteria counts in dairy calves and adult dogs

Waste milk is not suitable for human intake; however, it contains essential nutrients that can support calf growth. About 22–62 kg of waste milk is wasted per cow yearly in the USA, leading to a drastic economic loss to the dairy industries. In fact, feeding raw non-saleable waste milk may pose serious risks towards the transmission of infectious pathogens, such as *Mycobacterium avium*, *Mycobacterium bovis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* sp., *Mycoplasma*, and *Campylobacter* sp. Currently, non-medicated milk replacer (NMR) is widely used to replace whole milk for dairy calf feeding and is manufactured using vitamins, antibiotics, coccidiostats, probiotics, and ionophores as additives to support calf growth enormously.

Probiotic supplementation may induce the intestinal and ruminal microorganisms, and reduce the enteropathogens. Probiotics confer a health benefit to cows and dogs by regulating microbial homeostasis in the intestine, stabilizing the gastrointestinal barrier, producing bacteriocins, boosting immune system, and interfering with the potentiality of pathogens to bind to the intestinal wall. Moreover, the normal composition of the intestinal microflora in dogs can be altered by hectic conditions, such as oral administration of antibiotics, gastrointestinal infections, dietary changes, and weaning. Previous study demonstrated that a combination of *B. amyloliquefaciens* and *E. faecium* decreases the concentration of *Clostridium* sp. The ability of *B. amyloliquefaciens* to survive through the digestive process, germination within the digestive tract, and defecation via faecal matter makes it the most promising probiotic bacterium. *B. amyloliquefaciens* helps boosting the efficiency of absorption and digestion of nutrients by producing extracellular enzymes like cellulase, proteases, metalloproteases, and amylases. Furthermore, bacteriocins produced by *B. amyloliquefaciens* exhibit anti-pathogenic activity. In recent times, a number of studies have established that dietary supplementation of *B. amyloliquefaciens* boost intestinal microflora, gut morphology, and nutrient digestibility, thereby enhancing the growth rate and feed efficiency. These positive effects indicated that *B. amyloliquefaciens* could also improve the health status of calves and dogs.

In this investigation, 2 experiments were carried out to evaluate the bactericidal impacts of *B. amyloliquefaciens* CECT 5940 on the shedding of faecal pathogenic bacteria in dairy calves (Experiment 1) and in adults dogs (experiment 2). In experiment 1, a completely randomized design was used to investigate the faecal bacteria profile of Holstein dairy calves fed with either pasteurized waste milk (PWM; n = 9) or a formulated NMR (n = 9) for 60 d. The NMR was supplemented with active probiotic *B. amyloliquefaciens* CECT 5940. In experiment 2, addition of *B. amyloliquefaciens* CECT 5940 was carried out in two stages. The first stage started from day 7–37, and the second from day 44–71. The assessment of faecal score was measured on day 22, 37, 42, 57, 71, and 77 to determine the texture of the stools. Calves received PWM consumed ($P < 0.05$) more starter feed between day 16 and day 45. The calves fed NMR had more moisture faeces and less cough reflux than the PWM-calves. Feeding NMR to calves increased faecal *Klebsiella oxytoca* and *Proteus vulgaris* counts in comparison to PWM-calves. The administration of *B. amyloliquefaciens* CECT 5940 to the dog diet has no significant effect on the hardness of the stool. Meanwhile, bacilli count increased while coliforms count decreased upon *B. amyloliquefaciens* CECT 5940 administration, thereby revealing that *B. amyloliquefaciens* CECT 5940 survived the gastrointestinal passage

and rapidly colonized the dog intestine, which could positively affect the metabolism and composition of the intestinal microflora. Findings showed the auspicious role of *B. amyloliquefaciens* CECT 5940 as anti-pathogenic agent against the intestinal pathogenic bacteria present in dairy calves and adult dogs. The overall results indicated that NMR containing *B. amyloliquefaciens* CECT 5940 could be used as a replacement product to substitute PWM and improve the shedding of intestinal bacteria without affecting calves and dogs' health.

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